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^{1}H-Nuclear Magnetic Resonance Metabolic Profiling of Pantaneiro and Curraleiro Beef Breeds: Unveiling Adaptation Mechanisms in Brazilian Livestock

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ABSTRACT

This study provides the first metabolomic characterization of serum from Pantaneiro and Curraleiro beef cattle, unveiling their unique adaptations to the Pantanal and Cerrado biomes of Brazil. Blood was collected during the summer season to capture metabolic responses to extreme environmental conditions, including high temperatures and varying humidity. Metabolomics on serum samples were analyzed using nuclear magnetic resonance (^{1}H-NMR) spectroscopy; the spectra were processed using Chenomx NMR Suite software v.10. The processed data were analyzed using partial least squares discriminant analysis to identify key metabolites, separating the two breeds. Forty-four metabolites were identified in serum samples collected from 27 animals (14 Pantaneiro in Pantanal Biome and 13 Curraleiro in Cerrado Biome), of which 33 exhibited significant differences between the breeds. Pantaneiro cattle exhibited elevated levels of betaine, 2-hydroxybutyrate, and glycolate, indicative of adaptations to oxidative stress, osmoregulation, and metabolic efficiency under the humid and flood-prone Pantanal biome. Conversely, Curraleiro cattle showed higher concentrations of valine, glutamate, acetate, and citrate, highlighting nutrient efficiency and energy metabolism, critical for survival in the nutrient-poor and arid Cerrado biome. Elevated lactate levels in both breeds suggest shared adaptations to hypoxic or high-energy demand conditions. This research reveals the biochemical underpinnings of the resilience of these breeds, providing additional knowledge about metabolic strategies for enhancing stress tolerance and productivity in extreme environments. The findings underscore the value of metabolomics for understanding livestock adaptation and contribute to the development of climate-resilient livestock systems.

1 | Introduction

Local livestock breeds play a crucial role in sustaining biodiversity, supporting resilient agricultural systems, and preserving genetic resources critical to adapting to environmental challenges. However, their survival is increasingly threatened by the widespread adoption of specialized high-output breeds, which has accelerated the alarming extinction rates of underutilized genetic resources (FAO 2007; Sponenberg 2020). In Brazil, two of the earliest cattle breeds introduced to the Americas, Curraleiro and Pantaneiro, exemplify the importance

of conserving local breeds. These cattle have developed unique adaptations over centuries, enabling them to thrive in some of the most extreme environments in the country.

The Curraleiro breed, a descendant of the first cattle introduced to the Americas, has adapted to the harsh conditions of the Cerrado and Caatinga biomes, regions characterized by high temperatures, low rainfall, and nutrient-poor vegetation (Carvalho et al. 2010, 2013). For over 500 years, this breed has undergone natural selection with minimal human intervention, resulting in significant genetic diversity and exceptional adaptability (Rocha-Silva et al. 2023). These traits make Curraleiro cattle an invaluable resource for sustainable livestock production in semiarid regions (Carvalho et al. 2022).

Similarly, the Pantaneiro breed, derived from European cattle, has evolved to thrive in the dynamic Pantanal biome, which alternates between extreme droughts and seasonal flooding (Issa et al. 2006). These cattle possess remarkable traits, such as the ability to graze submerged pastures, resistance to parasites, and resilience to extreme environmental conditions, making them well-suited for sustainable and organic livestock systems (Mazza et al. 1994; Sereno 2002; Dani and De Oliveira 2013). Additionally, their grazing practices support ecosystem biodiversity and contribute to environmental balance (Biazolli et al. 2020). Despite their ecological and economic significance, these breeds are endangered due to habitat loss, climate change, and genetic dilution caused by crossbreeding with commercial cattle (FAO 2007; Bieber et al. 2020).

The Cerrado and Pantanal biomes present some of the most challenging conditions for livestock worldwide. In contrast to temperate regions with consistent forage availability and mild climates, these biomes demand resilience and adaptation to extreme environmental pressures (Hofmann et al. 2021; Silva et al. 2022). Curraleiro cattle efficiently utilize native vegetation and tolerate high temperatures (Carvalho et al. 2022; Rocha-Silva et al. 2023), while Pantaneiro cattle endure prolonged droughts and graze submerged pastures without compromising health or productivity (Mariante et al. 2009; Barbosa et al. 2013). These adaptations highlight the global importance of studying local breeds in extreme environments, particularly as climate change increasingly imposes similar challenges on agricultural systems globally.

Emerging technologies, such as metabolomics, provide a promising avenue to deepen our understanding of these adaptations. Metabolomics comprehends the analysis of metabolites within biological systems and offers insights into the biochemical pathways driving traits such as disease resistance, heat tolerance, and productivity (Sun et al. 2015; Guillemin et al. 2016; Yue et al. 2020). Applying metabolomics to breeds like Curraleiro and Pantaneiro can uncover the metabolic signatures linked to their resilience, supporting selective breeding and conservation programs.

Notably, while these breeds have been studied for their genetic diversity and adaptation, their metabolomic profiles remain unexplored. In this line of reasoning, utilizing nuclear magnetic resonance (^{1}H-NMR) spectroscopy to analyze the serum metabolome of Pantaneiro and Curraleiro cattle can reveal the physiological mechanisms behind their resilience to extreme environments. These insights could advance genetic selection, conservation strategies, and the development of climate-resilient livestock systems (Sereno 2002). By characterizing their metabolomic profiles, this study seeks to enhance our understanding of livestock adaptation, supporting future research on biodiversity preservation in agricultural systems.

2 | Materials and Methods

This study was carried out through a collaboration between the Federal University of Grande Dourados (UFGD, Dourados, MS, Brazil) and the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ-USP, Pirassununga, SP, Brazil) under the approval of the Ethics Committee on Animal Use from the Federal University of Grande Dourados (protocol #23/2015–03).

2.1 | Animals and Experimental Design

The animals in this study were raised on natural pastures with intermittent feed availability during the summer season in Brazil (December to March). Their primary feed source was *Brachiaria brizantha*, a grass native to the Cerrado biome. No supplemental feed was provided, ensuring that the animals' diet consisted entirely of pasture grazing under natural conditions. The study site was located in a region characterized by high temperatures, averaging 37°C during the summer, and high humidity levels exceeding 80% (as shown in Figure 1). These environmental conditions are typical of the Pantanal and Cerrado biomes during this time of year, exposing the animals to seasonal climatic stressors.

The study included 27 animals, comprising 13 Curraleiro and 14 Pantaneiro cattle. Blood samples (10 mL) were collected via jugular vein puncture using vacuum tubes without anticoagulants (BD Vacutainer, São Paulo, SP, Brazil) in the beginning of the summer season (January) with no prior fasting of the animals. Samples were centrifuged at 3000 × g for 15 min at 4°C to separate the serum. The resulting supernatant serum was transferred into labeled 2 mL plastic tubes and stored at −80°C until further analysis.

2.2 | Sample Preparation and NMR Spectra Processing

Serum metabolites were extracted following the protocol described by Cônsolo et al. (2021) with minor modifications. Briefly, serum samples were thawed at room temperature for 30 min in batches. Subsequently, 500 µL of serum was transferred into 2.0 mL Eppendorf tubes. To each tube, 480 µL of ice-cold acetone and ice-cold methanol were added, and the mixture was vortexed for 10 s. Following this, 400 µL of ice-cold chloroform was added, and the samples were vortexed again. The samples were then left on ice for 10 min before being centrifuged at 10,000 × g for 10 min at 4°C to precipitate proteins. Finally, the supernatant was collected in a new conical tube. For all samples, an aliquot was vacuum centrifuged overnight to remove the solvent (SpeedDry RCV 2–18 CD plus, Christ; Osterode am Harz, GER).

Samples were resuspended in 500 µL of phosphate buffer (pH 7.4) prepared in D_2O, containing 0.5 mM of 2,2-dimethyl-2-silapentane-5-sulfonate-d_6 (DSS-d_6) as an internal standard.

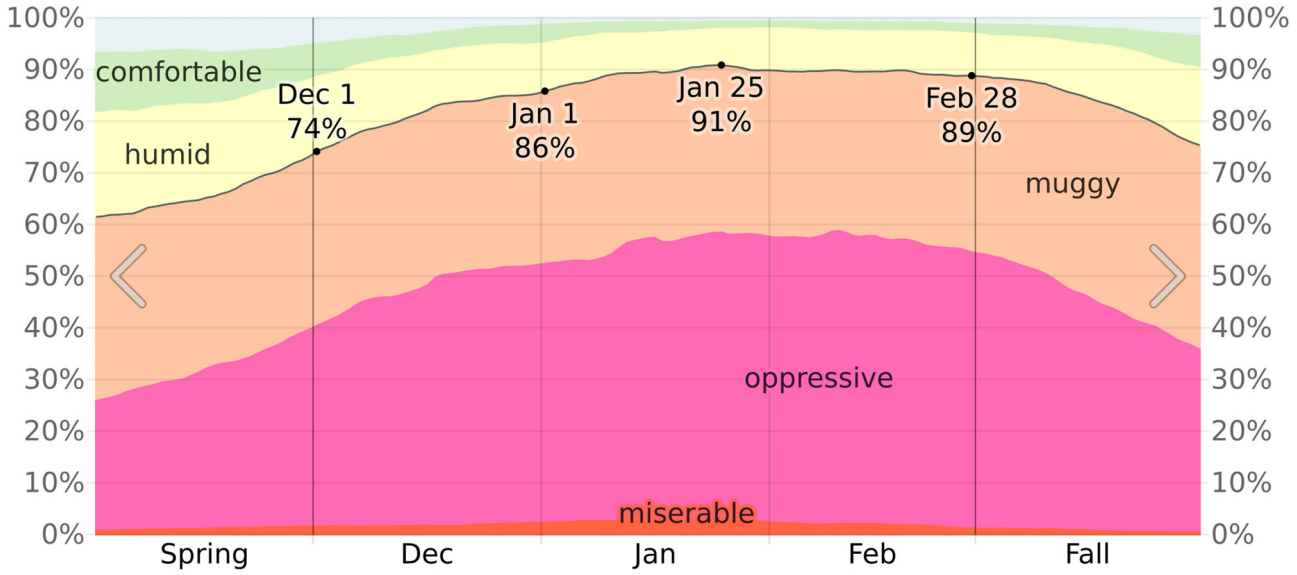

FIGURE 1 | Recorded humidity levels during December, January, and February in Dourados, Mato Grosso. The black dots show the recorded air humidity on each of the related days of the summer months.

The mixture was vortexed briefly and centrifuged at 14,000 × g for 5 min. Subsequently, the supernatant was carefully transferred to 5 mm NMR tubes and placed in the NMR spectrometer for analysis.

^{1}H-NMR spectra were recorded using a Bruker AVANCE III HD 600 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at 14.1 T, with ^{1}H observed at 600.13 MHz, equipped with a Broadband Observe (BBO) of 5 mm probe featuring z-gradient capabilities. All measurements were performed at 298.15 K, following the protocol described by (Da Costa et al. 2019). To suppress the residual water signal, the noesygppr1d pulse sequence was employed, with water signal suppression by irradiation at 2824.5 Hz (O1). Each ^{1}H NMR spectrum consisted of 128 scans, with the following acquisition parameters: spectral width (sw) of 12 019 Hz (20.0276 ppm), 90° pulse (P1) of 13.1 μs, acquisition time (aq) of 4.50 s, relaxation delay (d1) of 25s, data points (TD) of 108 170 (106 K), mixing time (d8) of 5 ms, and dummy scans (ds) of 4.

Spectra processing was carried out using TopSpin 3.7 software (Bruker). ^{1}H NMR chemical shifts were calibrated relative to the DSS signal at 0.00 ppm. Exponential line broadening of 0.3 Hz was applied before Fourier transformation. Automatic phase correction and baseline adjustment were performed, with manual correction applied when necessary to ensure spectral quality.

2.3 | Metabolite Identification and Quantitation

Metabolites in the 1D ^{1}H-NMR spectra were identified using the Chenomx NMR Suite Professional v10 software (Chenomx Inc., Edmonton, AB, Canada). Phasing and baseline correction were applied to ensure optimal spectral quality. All spectra were referenced to the methyl peaks of DSS at 0.00 ppm, which also served as an internal standard for quantitation.

A total of 44 metabolites were identified and quantified from the 1D ^{1}H-NMR spectra of serum extracts using the Profiler module of the Chenomx NMR Suite. This software utilizes an in-built 1D spectral library for precise metabolite identification. Quantitation was performed by comparing the area of selected metabolite peaks to the area under the DSS methyl peak, which represented a known concentration of 0.5 mM in all samples.

2.3.1 | Data Analysis

Data analysis was performed using R statistical software (version 4.3.0). Initially, data normalization was conducted using the Pareto scaling method implemented in the IMIFA package (version 2.1.10). Subsequent analyses were carried out using the mixOmics package (version 6.24.0), which facilitated sparse partial least squares discriminant analysis (SPLS-DA), partial least squares discriminant analysis (PLS-DA), and regularized canonical correlation analysis (RCC). To evaluate the significance of the PLS-DA model, the MVA.test function from the RVAideMemoire package (version 0.9–83) was used to perform permutation testing and compute *p*-values.

A matrix containing the concentrations of 44 metabolites across 27 samples (14 Pantaneiro and 13 Curraleiro cattle) was prepared, where each row corresponded to a sample and each column to a metabolite. SPLS-DA was employed to test the differences between the two breeds. This algorithm selects a fixed number of variables for each latent variable based on a predefined criterion (e.g., least absolute shrinkage and selection operator (LASSO) regression threshold). In this study, a fixed number of variables was used, and the model was optimized to identify variables that maximized prediction performance.

The SPLS-DA model required the optimization of two parameters: (1) the number of latent variables and (2) the number of variables retained for each latent variable. Crossvalidation was performed to optimize these parameters. Latent variables ranged from 1 to 16, and the number of variables per latent variable ranged from 4 to 20. During crossvalidation, samples from each class were randomly divided into calibration and validation sets, ensuring that all samples appeared in the validation set at least once. The calibration set was used to train the SPLS-DA model,

which was then tested on the validation set. This process was repeated five times, and the model with the highest classification accuracy was selected.

To validate the final model, permutation testing was conducted by refitting the model 2000 times with randomized class labels. This approach ensured that the observed classification accuracy was not due to random chance. The selected variables and their combinations were compared with those from the original SPLS-DA model to confirm the robustness of the results.

RCC was applied to identify similarities in metabolite profiles between the two breeds. Metabolites not included in the SPLS-DA results were used as input for this analysis. RCC estimated canonical vectors that represented metabolite profiles shared between the two groups. The penalty parameter for RCC was determined using the full set of metabolites absent from SPLS-DA, ensuring an accurate representation of shared metabolic features.

Additionally, clustered image maps (CIMs), derived from SPLS-DA, were used to visualize the separation between the two cattle breeds and to identify key metabolites contributing to the classification. These metabolites were further analyzed using PLS-DA to highlight breed-specific disparities. The PLS-DA results revealed 33 metabolites that differentiated the Pantaneiro and Curraleiro breeds.

3 | Results

A total of 44 metabolites were identified and quantified from the serum samples of cattle (Table 1). The SPLS-DA analysis identified 33 metabolites that significantly differed between the breeds. These included 2-hydroxybutyrate, 2-oxoglutarate, acetate, betaine, creatinine, dimethyl sulfone, galactarate, glucose, glycolate, hippurate, lactate, leucine, pyridoxine, sarcosine, 3-hydroxybutyrate, 3-phenylpropionate, citrate, creatine, acetone, alanine, alloisoleucine, glutamate, glycine, histidine, methanol, phenylalanine, pyruvate, tyramine, valine, glutamine, methionine, tryptophan, and propionate. These metabolites were critical in distinguishing between the breeds, as illustrated by the CIM in Figure 2.

Building on the data set of the 33 breed-specific metabolites identified via SPLS-DA, PLS-DA was performed to further explore patterns and differences within the data set. This analysis highlighted inherent metabolic variations between the breeds, which were visualized using PLS-DA confidence ellipses (Figure 3).

Using the dataset comprising 33 distinct metabolites obtained through SPLS-DA, the PLS-DA methodology was employed. This approach facilitated the investigation of inherent patterns and differences within the dataset (Figure 3, PLSDA confidence ellipses). Subsequently, novel CIMs were generated, wherein the most optimal demarcation ratio observed was 1:4 (Figure 4).

To elucidate the similarities between groups, RCC was employed (Figure 5). Notably, a prominent similarity was observed in metabolite concentrations, particularly for *isoleucine*. The Arrowplot visualization method was utilized to depict these correlations and distinctions, effectively. This approach represents shifts in the positions of two points, with the distances between them indicating the degree of similarity. Variables in closer proximity signify stronger correlations, whereas greater distances denote more pronounced dissimilarities.

Figure 6 shows the number of samples classified correctly as a function of the number of latent variables and the number of variables per component. The model with five latent variables and nine variables per latent variable showed the best performance. The assessment of model around this point showed that model with 4 latent variables and 10 variables were the best model. The permutation assessment (Figure 7) showed the validity of this model, where there was not a single observation where selected variables in the permutated models matched those from selected model.

4 | Discussion

This study presents the first metabolomic characterization of serum from Pantaneiro and Curraleiro cattle, offering new insights into their metabolic profiles and adaptations to contrasting environmental conditions. These findings advance our understanding of the physiological mechanisms underlying the remarkable resilience of these breeds, which have evolved to thrive in extreme environments. A total of 44 metabolites were identified, and 33 showed significant differences between the two breeds, highlighting distinct metabolic adaptations shaped by their specific biomes.

Pantaneiro cattle exhibited higher serum lactate levels (2.86 mM) compared to Curraleiro (1.97 mM), both exceeding the typical reference range for healthy cattle of 0.5—1.0 mM (Pereira et al. 1999; Burgos et al. 2024). Lactate plays a critical role in energy production during hypoxic or stressful conditions, such as heat stress or oxygen limitations (Meléndez et al. 2021). Higher lactate levels have been observed in cattle subjected to heat stress, prolonged lairage, or transport stress, often linked to anaerobic metabolism triggered by elevated energy demands and limited oxygen availability (Sullivan et al. 2024). Additionally, increased lactate concentrations are associated with microbial activity in heat-stressed cows, where ruminal lactate production is driven by an abundance of lactate-producing bacteria such as *Streptococcus* and unclassified *Enterobacteriaceae* (Kim et al. 2022a). These elevated lactate levels in Pantaneiro and Curraleiro breeds may suggest an adaptive metabolic response to the extreme conditions of the Pantanal and Cerrado biomes, characterized by high temperatures and variable forage availability.

Distinct breed-specific metabolites further highlight their unique adaptations. Curraleiro cattle, adapted to the nutrient-poor and arid Cerrado biome, exhibited higher levels of valine, glutamine, glutamate, and citrate. Valine and glutamate play key roles in protein synthesis, gluconeogenesis, and energy metabolism, enabling efficient nutrient utilization in nutrient-scarce environments (Howell et al. 2001). Elevated acetate and citrate levels suggest shifts in energy metabolism, particularly in the tricarboxylic acid (TCA) cycle, likely as a response to heat stress. This aligns with findings from Kim et al. (2022b), who reported heat stress-induced alterations in the TCA cycle.

Also, Pantaneiro cattle, thriving in the humid and flood-prone Pantanal biome, showed elevated levels of betaine, 2-hydroxybutyrate, and glycolate, reflecting adaptations to

TABLE 1 | Metabolites identified on serum samples of Pantaneiro and Curraleiro breeds.

	Pantaneiro				Curraleiro			
Metabolites	**Average**	**SD**[a]	**Max**	**Min**	**Average**	**SD**[a]	**Max**	**Min**
2-hydroxybutyrate	0.0191	0.0126	0.0386	—	0.0187	0.0102	0.0433	0.0066
2-oxoglutarate	0.0352	0.0226	0.0796	0.0036	0.0512	0.0268	0.1215	0.024
3-hydroxybutyrate	0.1909	0.1071	0.3271	0.0197	0.1973	0.0643	0.3021	0.0664
3-phenylpropionate	0.0203	0.0114	0.0403	0.0063	0.0154	0.0119	0.04	0.002
4-aminobutyrate	0.0368	0.0293	0.0786	—	0.0423	0.0180	0.0658	0.01
Acetate	0.7385	0.3988	1.535	0.0554	0.5334	0.2505	1.2106	0.316
Acetone	0.0305	0.0186	0.0619	0.0042	0.0358	0.0124	0.0625	0.0113
Alanine	0.1645	0.0851	0.3332	0.0068	0.1660	0.0391	0.2226	0.077
Alloisoleucine	0.0315	0.0248	0.0769	—	0.0404	0.0266	0.0807	0.0026
Betaine	0.0718	0.0351	0.1157	0.0017	0.0544	0.0325	0.1353	0.0067
Butyrate	0.0120	0.0064	0.0243	0.0023	0.0162	0.0095	0.0391	0.0022
Carnitine	0.0158	0.0117	0.0484	0.003	0.0197	0.0080	0.0397	0.0056
Citrate	0.0811	0.0613	0.1798	—	0.0636	0.0247	0.1039	0.0222
Creatine	0.0973	0.0523	0.1907	0.0049	0.1081	0.0227	0.1424	0.0516
Creatine phosphate	0.0365	0.0316	0.122	—	0.0423	0.0159	0.0743	0.0175
Creatinine	0.0062	0.0054	0.018	—	0.0105	0.0063	0.0268	0.0042
Dimethyl sulfone	0.0187	0.0113	0.0417	—	0.0271	0.0125	0.0515	0.0102
Formate	0.0868	0.0236	0.1276	0.0496	0.0985	0.0234	0.137	0.0415
Galactarate	0.0066	0.0059	0.0213	—	0.0084	0.0111	0.0417	0.0006
Glucose	1.0454	0.5132	1.7478	0.0877	1.1960	0.2950	1.7318	0.5261
Glutamate	0.0959	0.0848	0.3545	0.0057	0.1438	0.1027	0.3935	0.0193
Glutamine	0.0371	0.0250	0.1006	0.002	0.0531	0.0294	0.1072	0.0063
Glycine	0.2473	0.1259	0.4653	0.0107	0.2486	0.0641	0.3213	0.1177
Glycolate	0.0477	0.0375	0.1257	—	0.0324	0.0209	0.073	—
Hippurate	0.0686	0.0486	0.1876	0.0053	0.0462	0.0141	0.0734	0.0277
Histidine	0.0360	0.0200	0.0648	0.0058	0.0406	0.0115	0.0542	0.0179
Isobutyrate	0.0125	0.0129	0.053	0.0026	0.0118	0.0086	0.0349	0.0021
Isoleucine	0.0499	0.0367	0.1392	0.0021	0.0602	0.0337	0.1133	—
Isovalerate	0.0075	0.0040	0.0143	0.0014	0.0096	0.0056	0.0192	0.0031
Lactate	2.8615	2.0281	7.2646	0.0959	1.9712	0.7312	2.7421	0.3186
Leucine	0.0378	0.0284	0.0819	0.0023	0.0575	0.0221	0.1003	0.0276
Malonate	0.0170	0.0186	0.0508	—	0.0218	0.0149	0.0647	0.0104
Methanol	0.0985	0.2189	0.8538	—	0.1948	0.2682	0.7565	0.027
Methionine	0.0168	0.0114	0.0355	—	0.0192	0.0075	0.0269	—
Phenylalanine	0.0278	0.0211	0.0629	—	0.0368	0.0174	0.0667	0.009
Propionate	0.0471	0.0262	0.0905	0.0001	0.0541	0.0485	0.1758	—
Pyridoxine	0.0137	0.0101	0.0367	0.0043	0.0131	0.0100	0.0341	—
Pyruvate	0.0205	0.0236	0.0857	0.0019	0.0285	0.0155	0.0643	0.0096
Sarcosine	0.0060	0.0043	0.0142	—	0.0097	0.0053	0.02	0.0028
Threonine	0.0479	0.0342	0.1121	0.0028	0.0527	0.0202	0.0839	0.0218
Trimethylamine N-oxide	0.0328	0.0218	0.0835	0.0026	0.0350	0.0212	0.0862	—
Tryptophan	0.0205	0.0091	0.0354	0.0035	0.0243	0.0122	0.0496	0.0112

(Continues)

TABLE 1 | (Continued)

Metabolites	Pantaneiro				Curraleiro			
	Average	SD[a]	Max	Min	Average	SD[a]	Max	Min
Tyramine	0.0297	0.0215	0.0702	—	0.0328	0.0162	0.054	—
Valine	0.1164	0.0639	0.2403	0.0052	0.1516	0.0603	0.2564	0.0738

[a]Standard deviation.

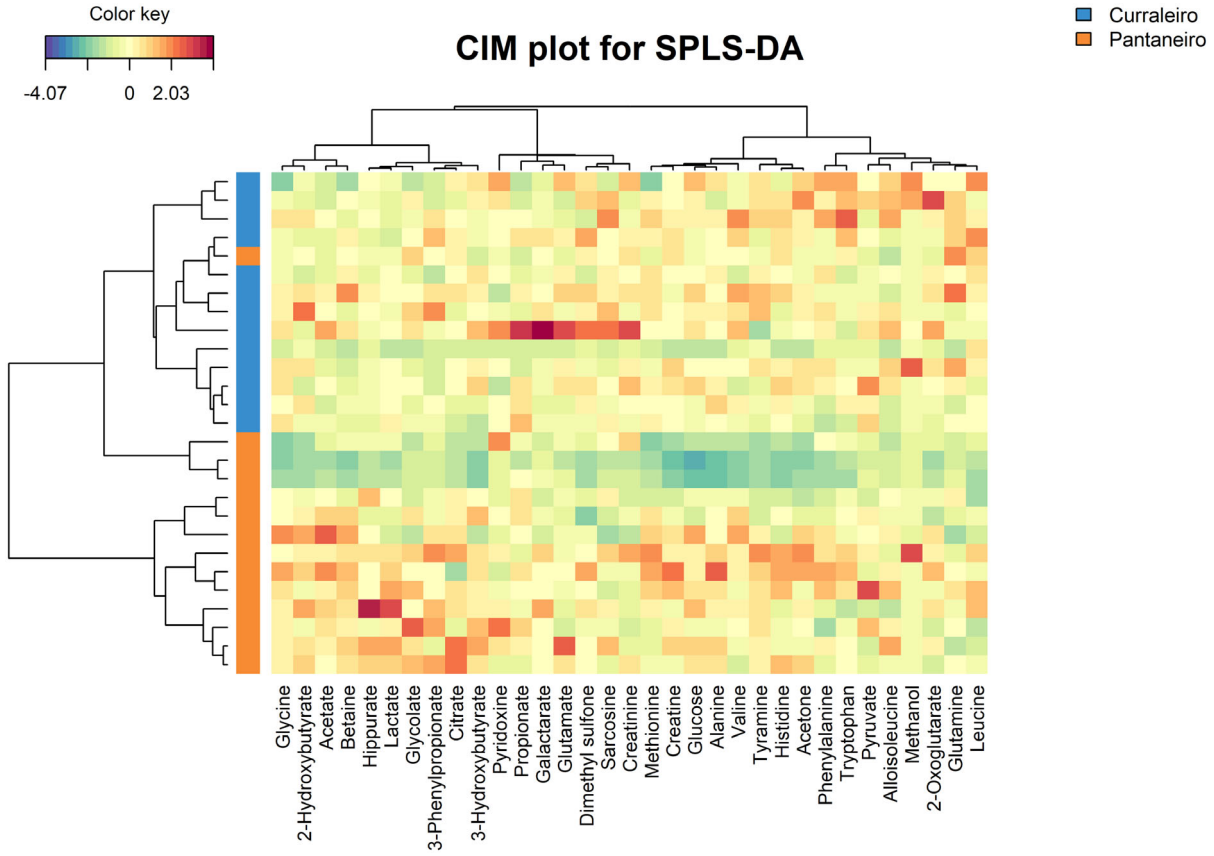

FIGURE 2 | CIMs derived from SPLS-DA showing the most predictive or discriminative metabolites to classify the samples. CIM = clustered image maps; SPLS -DA = sparse partial least squares discriminant analysis.

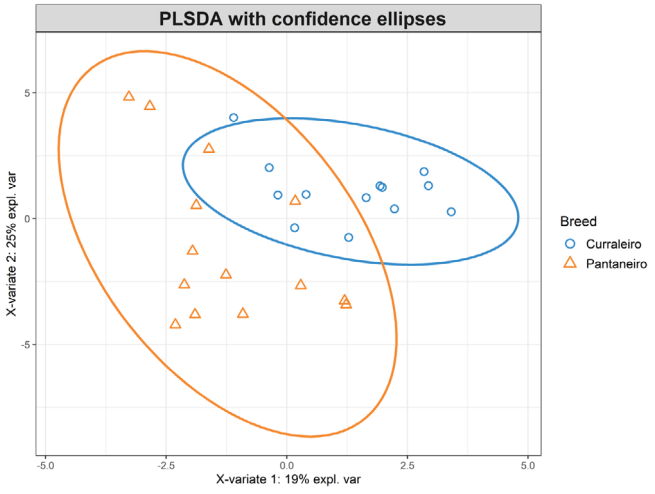

FIGURE 3 | PLS-DA scores plot showing confidence ellipses (95%) with Pantaneiro and Curraleiro breeds PLS-DA = partial least squares discriminant analysis.

oxidative and osmotic stress. Betaine plays a key role in osmoregulation and methylation, supporting metabolic homeostasis during stress (Eklund et al. 2005). Similarly, 2-hydroxybutyrate, a product of fatty acid metabolism, has been associated with oxidative stress regulation and metabolic efficiency (Cônsolo et al. 2021). Elevated creatinine levels in Pantaneiro cattle may reflect increased muscle metabolism or reduced renal function under dehydration caused by heat stress. This is consistent with findings that heat stress induces protein oxidation, leading to muscle proteolysis and elevated plasma urea and creatinine levels (Kekana et al. 2018; Greenwood 2021). Additionally, heat stress can impair kidney function, reducing creatinine clearance and exacerbating urea retention in circulation (Higashiyama et al. 2014). The higher concentrations of hippurate and 3-phenylpropionate in Pantaneiro cattle further highlight microbiota-driven adaptations to dietary and environmental pressures, as these metabolites are products of microbial fermentation and amino acid catabolism (Pallister et al. 2017; Zhu et al. 2023; Kim et al. 2024).

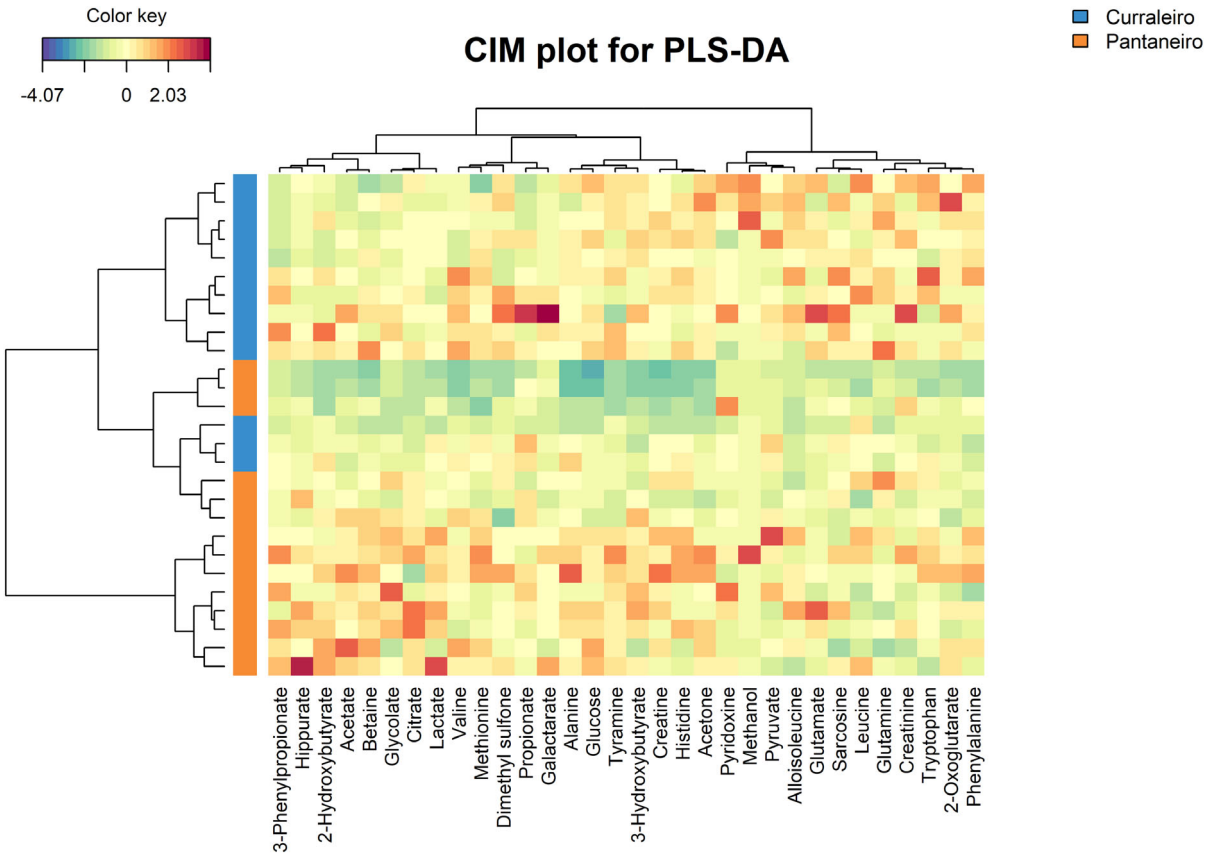

FIGURE 4 | CIMs of the PLS-DA model, showing the metabolites selected by SPLS-DA model. CIM = clustered image map; PLS-DA = partial least squares discriminant analysis; SPLS-DA = sparse partial least squares discriminant analysis.

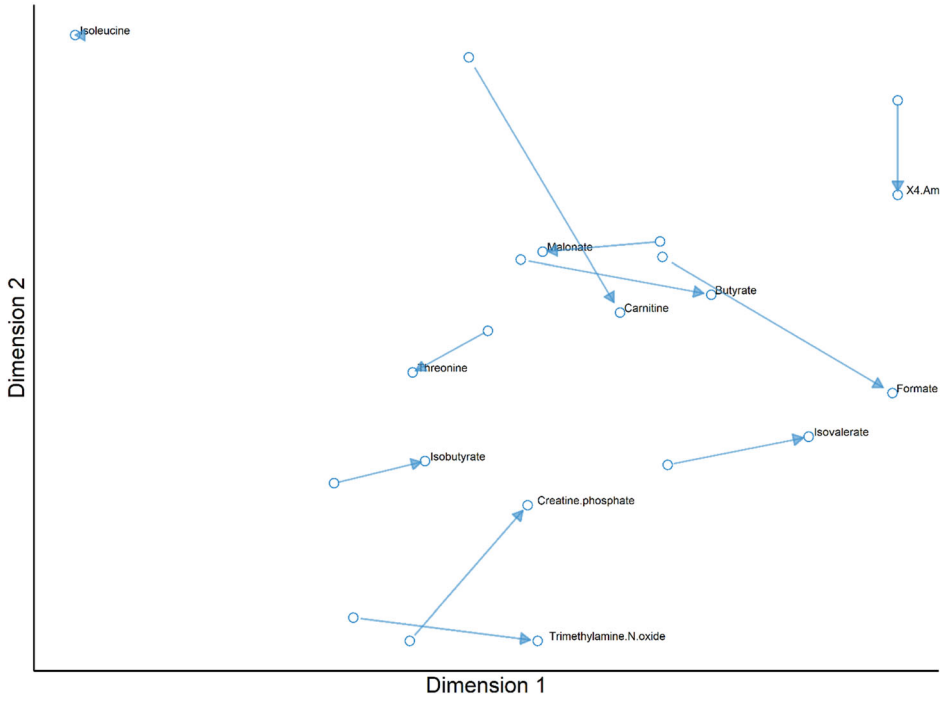

FIGURE 5 | Arrowplot showing the differences of selected metabolites between Pantaneiro and Curraleiro breeds. The shorter the arrow length is, the more similar the variables are in both datasets.

In Curraleiro cattle, a reduction in acetate concentrations was observed compared to Pantaneiro cattle. Under heat stress, the microbial community in the rumen undergoes significant shifts, leading to alterations in short-chain fatty acid production, typically characterized by a reduction in acetate production while increasing propionate and butyrate synthesis, which is more

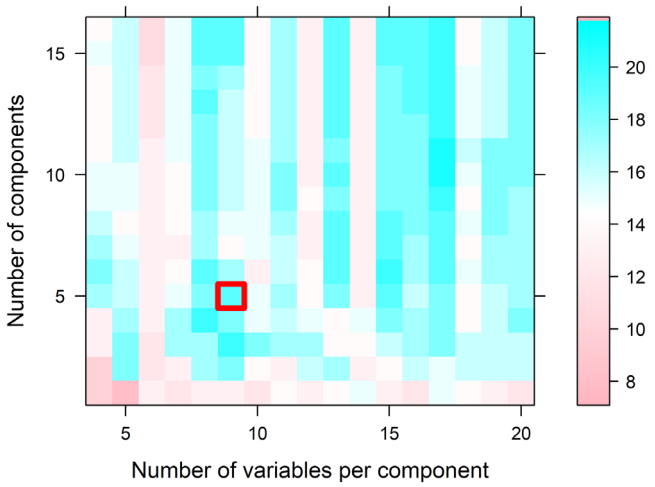

FIGURE 6 | Samples classified correctly as a function of the number of latent variables and number of variables per component.

energy efficient for animals (Nonaka et al. 2008; Kim et al. 2022b). This finding suggests that Curraleiro cattle may exhibit a more efficient adaptive response to heat stress, in which rumen microbial communities and fermentation pathways are optimized to enhance energy production under challenging environmental conditions. The observed differences in amino acid levels further underscore these adaptations. Curraleiro cattle exhibited higher levels of essential amino acids, such as leucine, alanine, and glutamine, which support energy production and gluconeogenesis. While heat stress typically reduces circulating amino acid concentrations due to increased glucose synthesis (Joo et al. 2021), the elevated levels in Curraleiro suggest a compensatory mechanism for protein catabolism under nutrient-limited conditions.

On the other hand, isoleucine emerged as a metabolite of interest due to its higher serum concentrations in Curraleiro compared to Pantaneiro cattle, while the arrowplot indicated a notable similarity in isoleucine concentrations between the breeds. Its presence has been linked to resilience in hypoxic conditions (Zhu et al. 2019) and to enhanced digestive efficiency through modulation of pancreatic enzymes (Cao et al. 2019). Additionally, isoleucine dynamically regulates metabolic responses to cold and heat stress in ruminants (Hu et al. 2021). These findings suggest that isoleucine serves as a conserved adaptive mechanism, reflecting its importance in supporting livestock resilience to environmental challenges.

These metabolite profiles reflect the interplay between genetic predispositions and environmental pressures. Pantaneiro cattle appear metabolically optimized for survival in a warm, humid biome where oxidative stress and periodic flooding demand resilience (Felix et al. 2013). Conversely, Curraleiro cattle have adapted to the arid Cerrado, prioritizing efficient resource utilization and energy conservation (Carvalho et al. 2013). Our findings offer practical implications for livestock management under climate change. Insights into the metabolic pathways that confer resilience in Pantaneiro and Curraleiro cattle could inform strategies to develop climate-adapted livestock globally.

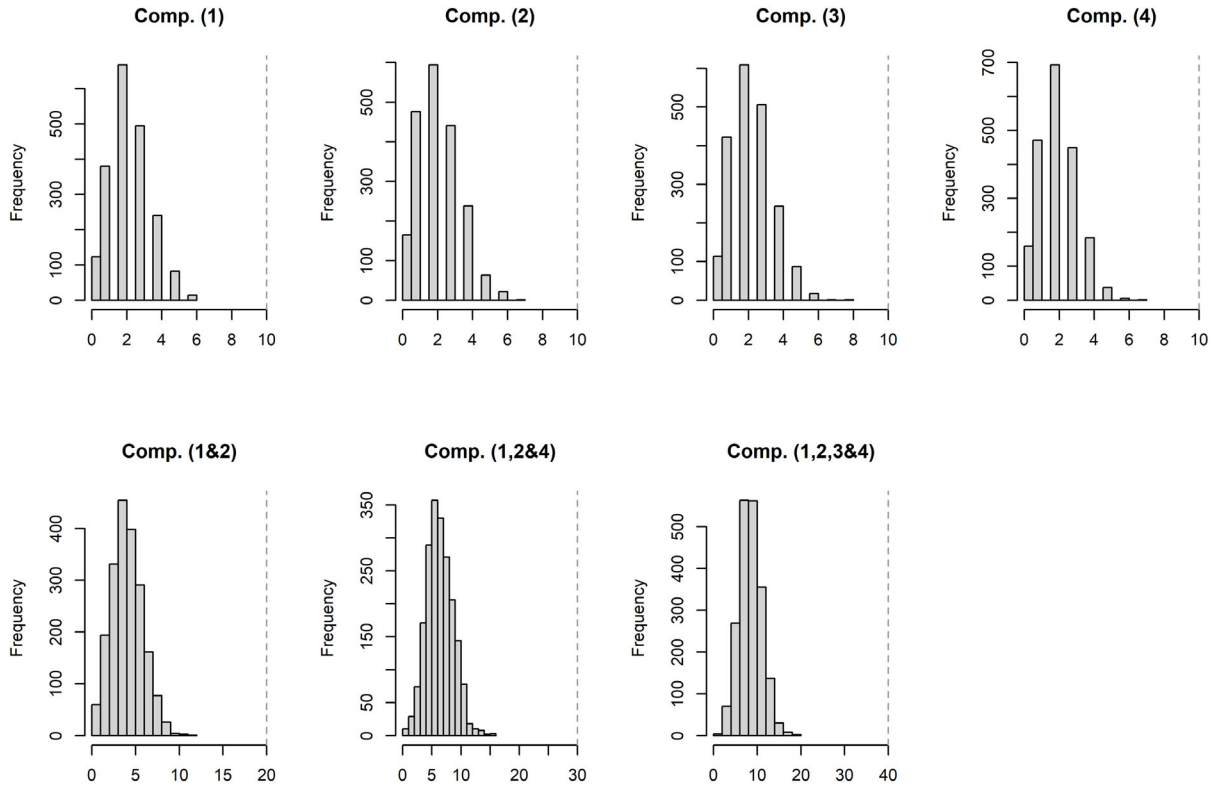

FIGURE 7 | Frequency of the number of variables that matched permutated and selected models. In the first row, figures are for single components, where a perfect match would mean 10 variables in the bottom axis. In the second row, figures are for sequential combination of components, where a perfect match would mean 20, 30, and 40 variables in bottom axis. The vertical dashed line corresponds to the expected number of variables with perfect match.

These breed-specific adaptations provide a framework for identifying metabolites expressed in those environmental conditions, which could be leveraged in breeding programs to enhance stress tolerance and productivity. Additionally, dietary interventions designed to optimize the availability of key metabolites may further enhance the adaptive potential of these breeds.

Future research should focus on integrating metabolomic data with genomic and transcriptomic analyses to uncover the genetic basis of these metabolic adaptations. Functional studies focusing on the roles of key metabolites in stress tolerance and longitudinal studies monitoring dynamic changes in metabolite profiles under environmental stressors would deepen our understanding of these adaptations. By exploring the metabolomic differences between Pantaneiro and Curraleiro cattle, this study provides a foundation for advancing sustainable livestock systems in extreme environments and underscores the value of conserving local breeds as reservoirs of genetic and physiological resilience.

5 | Conclusions

This study sheds light on the unique metabolic adaptations of Pantaneiro and Curraleiro cattle, providing a comprehensive understanding of their resilience to extreme environments. By identifying distinct metabolic signatures, this work highlights the complex interplay between genetics, environmental factors, and metabolic regulation in shaping adaptive traits. These findings contribute to the conservation and sustainable use of local breeds, offering valuable insights for selective breeding, livestock management, and climate resilience. Future studies should build on these results to further explore the mechanisms driving adaptation and their application in sustainable livestock systems.

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